Treatment outcomes in ANCA-associated vasculitis

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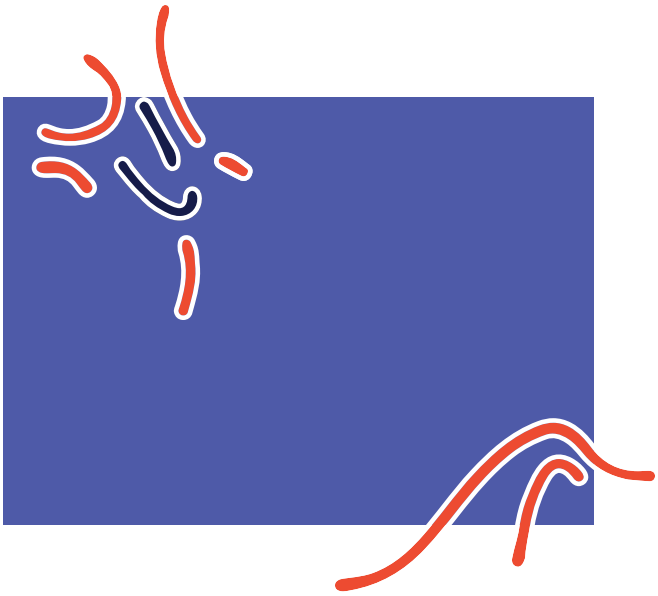
02 Chapter

Review: Gene variants and treatment outcomes in antineutrophil cytoplasmic antibody-associated vasculitis



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ABSTRACT

The introduction of immunosuppressive therapy for ANCA-associated vasculitis (AAV) has greatly improved outcomes, though patients now accumulate damage from vasculitis activity and adverse effects of treatment. Prediction of treatment outcomes using gene variants might help reduce this damage by allowing for personalized treatment. Several studies have studied genetic polymorphisms in relation to treatment outcomes of AAV. This review gives an overview of these studies, discussing both disease modifiers (influencing disease outcomes such as activity, severity and relapse risk) and pharmacogenetics (influencing drug metabolism and/or drug response). Subsequently, potential benefits of testing genetic variants for AAV and the steps needed for its implementation in clinical practice are discussed. The conclusion of this review is that measurement of most polymorphisms is currently not indicated in clinical practice, with the possible exception of thiopurine methyltransferase (TPMT), since homozygous carriers of TPMT variants have a strongly increased risk of severe bone marrow depression.

INTRODUCTION

Over the years, treatment of ANCA associated vasculitis (AAV) has greatly improved. After the introduction of combined therapy using cyclophosphamide (CYC) and high doses of glucocorticoids, it has turned from a lethal disease to a chronic relapsing-remitting condition with much improved patient survival [1,2]. Treatment toxicity was reduced by shortening CYC exposure and switching to azathioprine (AZA) maintenance therapy after stable induction of remission [3]. More recently, the monoclonal anti-CD20 antibody rituximab (RTX) was introduced, both as an alternative to CYC for induction therapy [4], as well as an alternative to AZA for maintenance therapy [5,6].

Despite these advances, patients still accumulate damage. This damage not only results from vasculitis activity, but also from adverse effects of treatment [7,8]. The balance between disease activity and treatment toxicity could potentially be improved using personalized therapy. Studying genetic variation in relation to disease and treatment outcomes might be an interesting approach to achieve this [9]. Some genetic factors predict individual handling and/or sensitivity to a drug (i.e., pharmacogenetics), possibly allowing for individual dosing to achieve maximum efficacy and minimum toxicity. Other genetic factors affect inflammatory pathways and may indirectly affect drug response. These factors might help in guiding drug choice [10].

This review will discuss gene polymorphisms studied in relation to outcomes of currently used treatment in AAV. Some of these polymorphisms are disease modifiers, as they are mainly involved with disease outcomes such as disease activity, severity and risk of relapse. Other polymorphisms are associated with pharmacogenetics, since they affect drug metabolism and/or response to drugs. Subsequently, the likelihood of and steps needed for practical usage of these polymorphisms to improve AAV treatment will be discussed.

Cyclophosphamide (CYC)

CYC has been the main drug used for induction therapy of AAV since its first introduction [1], and is still one of the primary options advocated for active AAV [6]. Unfortunately, CYC use is associated with adverse events such as leukopenia, infections, haemorrhagic cystitis and development of infertility and malignancies.

CYC is a prodrug that requires activation by cytochrome P (CYP)450 enzymes in the liver into the active metabolites 4-hydroxycyclophosphamide and aldophosphamide. Catalysts for this conversion include CYP2B6, CYP2C9, CYP2C19 and CYP3A4 [11]. Several studies performed in systemic lupus erythematosus patients found that the genetic variant CYP2C19*2 (681G>A; rs4244285) was associated with a reduced risk of ovarian toxicity [12-14], possibly at the cost of a reduced clinical response [12]. Two studies have investigated the clinical implications of CYP450 gene variants for ANCA associated vasculitis.

Schirmer et al. studied several gene variants of CYP2C9 and CYP2C19 in relation to treatment outcomes of 196 mostly Caucasian AAV patients treated with CYC. The presence of genetic variant CYP2C9*2 (430C>T; rs1799583) or CYP2C9*3 (1075A>C; rs1057910), as

was the case in 65 (33%) patients, was associated with a higher risk of leukopenia. This difference existed only in patients treated with oral (not intravenous) CYC, and carriers of a CYP2C9 variant (CYP2C9*2 or CYP2C9*3) treated with oral CYC also showed a trend towards a lower risk of refractory disease. CYP2C9*2 and CYP2C9*3 have been shown to result in a slower conversion of CYC to active metabolites [15,16]. This slower activation might result in prolonged exposure to active metabolites, with increased CYC efficacy and toxicity as a result. CYP2C19*2 (681G>A; rs4244285), present in 55 (28%) patients, was not related to clinical endpoints in this study [17].

In a study by Cartin-Ceba et al. based on data from the RAVE trial, including 93 patients treated with CYC, most frequently of Caucasian ethnicity, no associations were found for SNPs of CYP2B6 (1459C>T, rs3211371), present in 16 (17%) patients, or CYP2C19 (681G>A, rs4244285), present in 18 (19%) patients, with time to complete remission. Unfortunately, due to a relatively small sample size, effects of more uncommon SNPs (e.g., CYP2C9) could not be addressed in this study [18].
In conclusion, genetic variants of CYP2C9 may be associated with clinically relevant differences in efficacy and toxicity of CYC. Theoretically, CYP2C9*2 and CYP2C9*3 carriers should receive lower doses of oral cyclophosphamide to compensate for prolonged exposure to its active metabolites.

Azathioprine (AZA)
AZA is currently the main drug used for maintenance therapy of AAV, after disease remission has been attained [6]. The CYCAZAREM trial, published in 2003, showed that switching to AZA after induction of remission with CYC was equally effective as continued CYC therapy for prevention of relapse, allowing for reduced exposure to the cumulative toxic effects of CYC [3].

AZA is a prodrug that is converted enzymatically into 6-mercaptopurine (6-MP). Subsequently, it is converted into 6-thioguanine nucleotides (6-TGN), the active metabolite responsible for the immunosuppressive effects, by the enzyme hypoxanthine phosphoribosyltransferase (HPRT). Two competitive pathways exist that instead convert 6-MP to inactive metabolites. The most well-known is the enzyme thiopurine methyltransferase (TPMT), that converts 6-MP into 6-methylmercaptopurine (6-MMP). The other pathway, Xanthine Oxidase (XO), converts it into thiouric acid (TUA) instead [19]. Several SNPs of TPMT have been described, resulting in reduced activity of the enzyme. Reduced TPMT activity indirectly results in higher levels of 6-TGN [19,20]. Because of a strongly increased risk of myelosuppression resulting from these increased 6-TGN levels, it has been advised that patients homozygous for genetic variants of TPMT either start with a 10-fold reduced dose of AZA or use a different immunosuppressive drug [20]. Patients heterozygous for gene variants of TPMT are advised to start with a 30-70% reduced dose of AZA [20]. In an RCT performed in 2015, dose reduction in patients heterozygous for TPMT variants resulted in a strong reduction of adverse effects in this subgroup of inflammatory bowel disease patients, while maintaining efficacy. Because of the low frequency of TPMT variant carriers, with only 10% of patients in the intervention group requiring dose adjustment, no effect was shown in intention-to-treat analysis [21].

Two studies on TPMT genotype have been performed in AAV patients. Neither study found a significant association of TPMT genotype with adverse effects or efficacy of AZA therapy [22,23]. This indicates that TPMT pretesting might not be as useful for AAV patients as it is for other populations. However, the most recent study showed a trend towards a higher sensitivity to leukocytopenia in patients with lower TPMT activity, as well as a trend towards better relapse-free survival in patients with lower TPMT activity [23]. This suggests that there might be a small effect of TPMT genotype on AZA efficacy and toxicity, and that the sample size of these studies might simply be too small to detect these effects, in particular because of the low frequency (6-10%) of TPMT variant carriers. Of note, neither of these studies included patients that were homozygous for TPMT variants (prevalence approximately 0.3%) [22,23].

In conclusion, adjustment of initial AZA dose may be less relevant for AAV patients carrying one gene variant of TPMT, as long as dose is adjusted based on frequent blood count measurements. This is not to say that TPMT pretesting is irrelevant in AAV, since homozygous variant carriers (1 in 178 to 1 in 3,736 patients) have a strongly increased risk of severe myelosuppression from AZA [20].

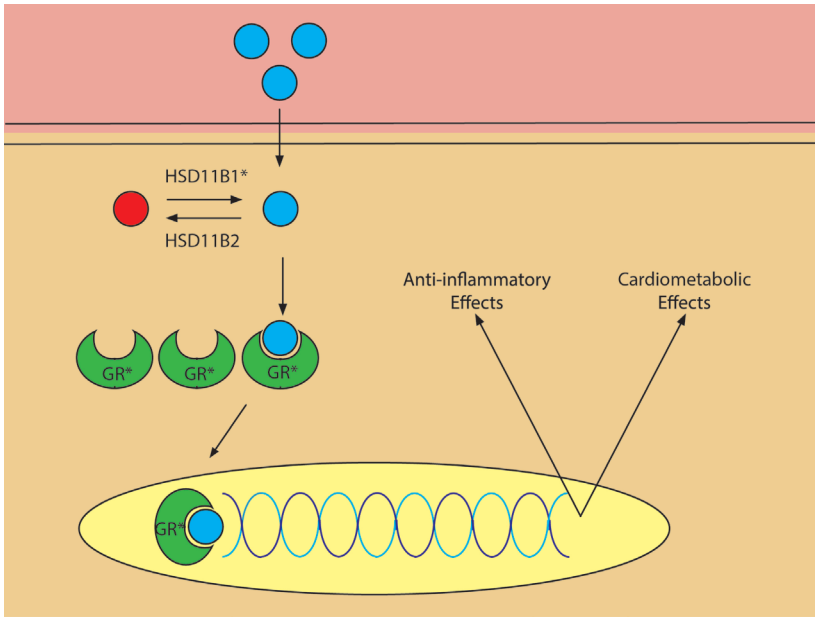
Prednisolone (PRED)

Glucocorticoids, usually PRED, form a standard part of induction and maintenance therapy for AAV [6]. Although effective, PRED treatment in AAV is associated with a wide range of adverse effects affecting, among others, inflammation, the cardiovascular system and glucose, lipid and bone metabolism. Some of these adverse effects contribute to treatment-related mortality [24-26]. A simplified scheme of glucocorticoid action is shown in **Figure 1**. Several gene polymorphisms have been described of the glucocorticoid receptor (GR) and 11β-Hydroxysteroid dehydrogenase type 1 (HSD11B1). To our knowledge, only one observational cohort study has been performed that related gene polymorphisms affecting glucocorticoid sensitivity to relevant clinical outcomes in AAV [27].

Glucocorticoid receptor (GR)

PRED, as well as the endogenous glucocorticoid cortisol, exert most of their effects through interaction with the GR, a member of the nuclear receptor family. This receptor binds its ligand in the cytoplasm and subsequently moves to the nucleus, where it affects gene expression. This results in a multitude of cardiovascular, metabolic and inflammatory effects [28]. Several functional variants of the gene encoding the GR have been identified. Some of them, including N363S (rs6195) and BclI (rs41423247), are associated with increased glucocorticoid sensitivity. Others, such as ER22/23EK (rs6189 and rs6190) and 9β (rs6198), are associated with glucocorticoid resistance [28]. Lastly, TthIII1 (rs10052957) is associated with glucocorticoid resistance mainly through linkage disequilibrium with ER22/23EK [29]. Haplotypes of the GR have been defined based on frequent combinations of SNPs [30]. Genetic variation in the GR has been linked to various cardiometabolic and inflammatory outcomes in the general population [31,32], as well as to disease severity and PRED response in inflammatory conditions such as multiple sclerosis and RA [33-37].

Figure 1. Simplified scheme of glucocorticoid action.



Cortisol/prednisolone (blue) enters a cell through the cell membrane. Subsequently, it can be converted to inactive cortisone/prednisone (red) by hydroxysteroid dehydrogenase 11β type 2 (HSD11B2), or reactivated by hydroxysteroid dehydrogenase 11β type 1 (HSD11B1). Cortisol/prednisolone binds the glucocorticoid receptor (GR) in the cytoplasm. After binding, the complex moves to the nucleus where it affects expression of genes related to inflammation, volume homeostasis and carbohydrate, lipid and protein metabolism. Gene polymorphisms of proteins marked with "" are discussed in this review.*

In an observational cohort study involving 241 patients from our center, two haplotypes of the GR were related to relevant clinical outcomes in AAV. Haplotype 4 (ER22/23EK+9β+TthIII1), present in 6% of patients, was associated with an increased risk of end-stage renal disease as well as an increased risk of mortality, suggesting that patients with this haplotype have a more severe disease phenotype. Homozygous carriers of haplotype 1 (BclI), entailing 6% of patients, had an increased risk of developing dyslipidemia, suggesting a less favourable metabolic phenotype in these patients. These differences existed despite similar glucocorticoid exposure in all haplotypes [27].

11β-Hydroxysteroid dehydrogenase type 1 (HSD11B1)

HSD11B1 is an enzyme present in most cells of the body that regulates local glucocorticoid levels. In vivo, it mainly converts cortisone to its active metabolite cortisol [38]. Based on findings from an earlier study, the A variant of rs11119328 SNP was hypothesized to be associated with reduced expression of HSD11B1 compared to the C variant [39].

In a cohort study of 241 AAV patients, the A variant of rs11119328 was associated with an increased risk of relapse in AAV despite similar glucocorticoid exposure to non-carriers of this variant, but only in non-carriers of Haplotype 1 of the GR. This combination exists in 19% of patients. These findings suggest that reduced local activation of glucocorticoids as a result of rs11119328 A results in a pro-inflammatory phenotype, but can be compensated for by increased sensitivity of the GR. Interestingly, glucocorticoid exposure did not differ between carriers and non-carriers of rs11119328 [27].

Overall conclusions on SNPs in PRED treatment

Two haplotypes of the GR (Haplotypes 1 and 4) and one SNP of HSD11B1 (rs11119328) were associated with relevant clinical outcomes of AAV in one study. After confirmation of the effects in an independent cohort, it might be interesting to investigate adjustment of treatment. For homozygous Haplotype 1 carriers this would entail reduction of glucocorticoid exposure, since these patients are apparently at increased risk of developing metabolic adverse events from PRED. For Haplotype 4 carriers, research on early aggressive treatment to prevent end-stage renal disease and subsequent mortality might be interesting. Carriers of rs11119328 without Haplotype 1 might benefit from prolonged maintenance therapy of glucocorticoids with or without another immunosuppressive drug, to compensate for the increased relapse risk when receiving standard treatment.

Rituximab (RTX)

RTX is a chimeric immunoglobulin 1 (IgG1) monoclonal antibody targeting CD20, present on B-cells. It is a relatively new drug for remission induction in AAV. The Rituximab in ANCA-Associated Vasculitis (RAVE) trial, published in 2010, showed it to be non-inferior to CYC [4,40]. More recently, the results of the Maintenance of Remission using Rituximab in Systemic ANCA-associated Vasculitis (MAINRITSAN) trial indicated that RTX might be superior to AZA for maintenance of remission after CYC induction [5,41].

As RTX is a monoclonal antibody targeting a specific inflammatory pathway, response to RTX is mainly influenced by genetic variation in its effector pathways rather than its metabolic pathway. Three studies have been performed investigating disease-modifying gene variants and their association with outcome of RTX treatment [18,42,43].

Fcγ receptor (FcγR)

RTX is capable of depleting B-cells through complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity (ADCC) by binding of its Fc fragment to Fcγ receptors (FcγR). The affinity of FcγR is affected by genetic variation and potentially influences RTX response. An FcγRIIIa SNP has previously been associated with clinical response to RTX in rheumatoid arthritis (RA) patients [44,45], as well as other auto-immune diseases [46,47].

In a study using data from the RAVE trial, which included 96 mostly Caucasian patients receiving RTX treatment, the 27 (28%) homozygous carriers of the FcγRIIa 519 AA genotype (rs1801274), associated with increased receptor affinity for IgG3 and capability of binding IgG2, had a shorter time to complete remission compared to the GG and GA variants. Interestingly, this association was independent of the induction treatment used, as it was also present in the CYC-treated group. The combination of FcγRIIa 519

(rs1801274) AA and FcγRIIIa 559 (rs396991) GG genotypes was associated with a higher frequency of disease remission, again irrespective of induction treatment used [18]. These results were in line with an earlier study showing that the combination FcγRIIa 519 GG + FcγRIIIa 559 TT was associated with an increased risk of relapse in GPA [48]. Based on the results of these studies, the FcγRs seem to play a role in AAV pathogenesis. One hypothesis is that genetic variation in FcγRIIa and FcγRIIIa affects the binding of ANCA to these receptors, resulting in increased ANCA clearance in those carrying FcγRIIa 519 GG + FcγRIIIa 559 TT [18,48]. Another hypothesis is that FcγRIIa 519 GG + FcγRIIIa 559 TT reduces clearance of staphylococcus aureus, resulting in chronic nasal carriage as a risk factor for relapse [48,49]. Alberici et al. did not find a relation of rs1801274 or rs396991 with treatment response, although they did not analyse homozygous variant carriers separately [42].

B cell activator of the tumor necrosis factor family (BAFF)

B cell activator of the tumor necrosis factor family (BAFF) is a cytokine relevant for B-cell development and survival. In auto-immune diseases, it is thought to promote inflammation by stimulating survival and proliferation of autoreactive B cells [50]. BAFF levels in AAV patients are increased compared to healthy controls. In contrast to other auto-immune diseases, BAFF levels showed no positive correlation with auto-antibody levels in AAV [51-53]. The rs9514828 SNP of BAFF has previously been associated with RTX response rate in RA [54].

In a European cohort of 213 AAV patients investing a panel of 18 candidate SNPs potentially associated with response to RTX, the B-cell activating factor (BAFF) SNP rs3759467 was associated with shorter time to relapse within 12 months [42]. This finding was confirmed in a replication cohort of 109 patients from the United Kingdom, but existed only in homozygous carriers of the C variant allele (n=7, or 2% of combined cohort). Besides a shorter time to relapse within 12 months after first RTX infusion, patients with the CC genotype of rs3759467 had a higher rate of detectable peripheral B-cells and less reduction of IgM levels compared to TC and TT genotypes. The association was not present in MPO-ANCA positive patients, although this might be due to the limited sample size of the study and the low frequency of homozygous variant carriers. The study did not show any association of other BAFF-related SNPs such as rs9514828 with clinical response in AAV. The exact mechanism behind the effects of the rs3759467 SNP are not yet clear. Most likely, the SNP affects B-cell survival, possibly through an increase in BAFF levels [42]. Hypothetically, patients with the rs3759467 SNP might benefit from addition of the monoclonal antibody belimumab, which blocks binding of BAFF to B-cell receptors [55].

Interleukin-6

Interleukin-6 (IL-6) is a cytokine with a wide range of functions. In the immune system, it is important for inducing the acute phase response and has a role in B-cell maturation and plasma cell proliferation. In auto-immune disease, it contributes to on-going inflammation through stimulation of immune cell proliferation and activation of lymphocytes. It also skews development of naïve T-cells towards T helper (T_H)17 cells rather than forkhead box protein 3 positive regulatory T (FOXP3+ T_{reg}) cells [56]. In RA, being homozygous for the -174 (rs1800795) C variant of the IL-6 promoter region has been associated with poor response to RTX [57].

In 2012, Robledo et al. conducted an observational study investigating the -174 (rs1800795) SNP in a Spanish Caucasian cohort of 144 patients with varying systemic auto-immune diseases, including 16 AAV patients. They found that homozygous carriers of the C variant of this SNP (9% of patients) had a significantly higher risk of non-response to RTX compared to carriers of CG and GG variants [43]. Alberici et al. did not find such an association in their study of 213 European AAV patients, although they did not analyse homozygous carriers of the C variant of rs1800795 separately [42].
The functional relation between this IL-6 SNP and RTX response is not entirely clear. A feasible hypothesis is that the reduced efficacy of RTX in -174 C homozygotes results from improved B-cell survival mediated by IL-6. An association of -174 genotype with serum IL-6 levels was not found, although a great inter-individual variation was noted [57]. Theoretically, -174 C homozygotes might benefit from addition tocilizumab to treatment, as this drug blocks the IL-6 receptor [58].

IL-2–IL-21 region

The rs6822844 SNP in the IL-2–IL-21 region on chromosome 4 has previously been associated with risk of autoimmunity and response to rituximab therapy [59]. IL-2 induces T-cell proliferation and promotes differentiation of regulatory T-cells while inhibiting Th17 differentiation and inducing activation-induced cell death [60]. IL-21 has a broad range of effects on the immune system, including both stimulatory and regulatory effects. Several auto-immune diseases have been associated with increased levels of IL-21 [61].

When specifically analysing MPO-ANCA positive patients(n=29), Alberici et al. found an association of SNP rs6822844 in the IL-2–IL-21 area (minor allele frequency 0.127) with risk of relapse within 6 months and time to relapse within 12 months [42]. The mechanism behind this might be that the G variant of rs6822844 promotes ADCC through IL2-mediated stimulation of natural killer (NK) cell proliferation and cytotoxicity [59,60]. The finding was not confirmed in a smaller replication cohort (n=19), although this could be due to a lack of statistical power, as there was a trend towards a better treatment response in carriers of the G variant of rs6822844 [42].

Overall conclusion on SNPs in RTX treatment

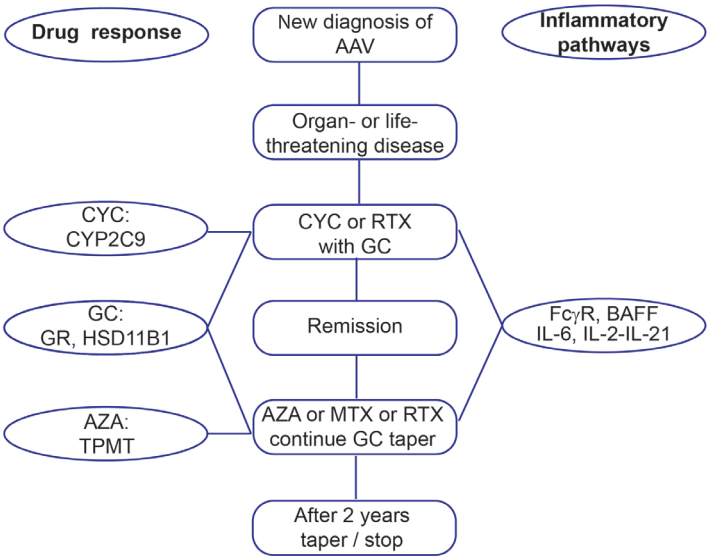
In conclusion, the SNPs rs1801274 (FcγRIIa), rs396991 (FcγRIIIa), rs3759467 (BAFF), rs1800795 (IL-6) and rs6822844 (IL-2 – IL-21) all appear to affect response to RTX treatment. All studied polymorphisms are related to effector pathways rather than drug metabolism, contrary to the gene variants studied in relation to CYC and AZA treatment. Therefore, these effects are not specific to RTX treatment and affect treatment efficacy more than toxicity. In some cases, such as SNPs related to BAFF and IL-6, it might be interesting to study whether patients with specific gene variants might benefit from addition of therapy specifically targeting the respective factor, such as belimumab (targeting BAFF signalling) and tocilizumab (targeting the IL-6 receptor). This would first require confirmation of increased BAFF or IL-6 signalling in patients with the respective genotypes.

Other genetic associations with outcomes of ANCA-associated vasculitis
To our knowledge, no studies have been performed in AAV patients of gene variants in relation to efficacy and toxicity of methotrexate or mycophenolate mofetil. Several other genetic variants, not related to a specific treatment, have been associated with clinical outcomes of AAV. Examples include gene polymorphisms of monocyte chemoattractant protein-1 (MCP-1) [62], alpha-1 antitrypsin [63] and human leukocyte antigen (HLA)-DPB1 [64]. Except for TPMT, none of the gene variants currently has practical relevance in AAV treatment.

DISCUSSION

Several observational studies have been performed regarding genetic factors associated with response to cyclophosphamide (CYC), rituximab (RTX), prednisolone (PRED) and azathioprine (AZA) therapy in AAV. An overview of proteins encoded by potentially relevant SNPs per drug used in AAV treatment is shown in **Figure 2**. The main results per study are summarized in **Table 1** and **Table 2**.

Figure 2. Treatment of ANCA-associated vasculitis and potential targets for personalized treatment



AZA azathioprine; BAFF B cell activator of the tumor necrosis factor family; CYC cyclophosphamide; CYP cytochrome P; FcγR Fcγ receptor; GR glucocorticoid receptor; HSD11B1 11β-hydroxysteroid dehydrogenase type 1; IL interleukin; MMF mycophenolate mofetil; MTX methotrexate; PRED prednisolone; RTX rituximab.

Table 1. Overview of drug metabolism-related gene polymorphisms in relation to clinical outcomes in ANCA-associated vasculitis

Drug/gene	Participants (ethnicity)	MAF	Efficacy	Toxicity	Reference
CYC					
CYP2C9	196 (96% Caucasian)	0.171	Trend: less refractory disease in carriers	Higher risk of leukopenia in carriers	[17]
CYP2C19	196 (96% Caucasian)	0.153	NS	NS	[17]
	93 (97% Caucasian)	0.108	NS	NS	[18]
CYP2B6	93 (97% Caucasian)	0.091	NS	NS	[18]
AZA					
TPMT	108 (Caucasian)	0.032	NS	NS	[22]
	207 (Caucasian)	0.046	NS	NS	[23]
PRED					
GR Haplotype 1	241 (Caucasian)	0.247	No increased relapse risk from HSD11B1	Homozygous: more dyslipidaemia	[27]
GR Haplotype 4	241 (Caucasian)	0.031	Higher risk of ESRD and mortality	NS	[27]
HSD11B1	241 (Caucasian)	0.190	Higher risk of relapse in carriers	NS	[27]

AZA azathioprine; CYC cyclophosphamide; CYP Cytochrome P; ESRD end-stage renal disease; GR glucocorticoid receptor; MAF minor allele frequency; NA not analysed; NS no significant differences; PRED Prednisolone; TPMT thiopurine methyltransferase.

Table 2. Overview of inflammation-related gene polymorphisms in relation to clinical outcomes in ANCA-associated vasculitis

Drug/gene	Participants (ethnicity)	MAF	Efficacy	Toxicity	Reference
BAFF	213 (Europe), 109 (UK)	0.172	Homozygous: higher risk of and shorter time to relapse	NA	[41]
Complement C1QA	213 (Europe), 109 (UK)	0.380	NS	NA	[41]
FCγR IIA	96 (93% Caucasian)	0.500	Shorter time to complete remission in homozygous	NA	[18]
	213 (Europe), 109 (UK)	0.495	NS	NA	[41]
FCγR IIB	213 (Europe), 109 (UK)	0.110	NS	NA	[41]
	96 (93% Caucasian)	0.104			[18]
FCγR IIIA	213 (Europe), 109 (UK)	0.439	NS	NA	[41]
	96 (93% Caucasian)	0.280	NS	NA	
IL-2-IL-21	213 (Europe), 109 (UK)	0.127	MPO-AAV: less relapse, longer time to relapse (not in UK cohort)	NA	[41]
IL-6	213 (Europe), 109 (UK)	0.359	NS	NA	[41]
	144 (Caucasian), 16 AAV	0.306	More non-response in homozygous carriers	NA	[42]
NFKB1	213 (Europe), 109 (UK)	0.383	NS	NA	[41]
TGFB1	213 (Europe), 109 (UK)	0.090	NS	NA	[41]

BAFF B cell activator of the tumor necrosis factor family; FcγR Fcγ receptor; IL-6 Interleukin-6; NA not analysed; NS no significant differences

While pharmacogenetics in the case of synthetic immunosuppressive drugs such as CYC, AZA and PRED involves mainly genetic variation in metabolic or functional pathways of these drugs, potentially allowing individual dosing of these drugs, genetic variants studied in relation to RTX response are mainly involved in inflammatory pathways. The latter may not necessarily be specific to RTX-treatment, as was seen for gene variants of the FcγR [18,48]. On the other hand, the inflammatory pathways identified might be interesting for targeted therapy, especially considering the large variety of targeted drugs currently available.

Theoretically, pretesting SNPs will help improve prediction of efficacy and toxicity of drugs used for AAV treatment. Treatment dose or modality could be adjusted in carriers of a genetic variant in order to optimize treatment outcomes for these patients. As a result, toxicity and healthcare costs would be reduced for these patients, especially considering the reduction of genotyping costs over recent years. Ideally, treatment modality and dose for the individual patient would become partly based on a panel of relevant SNPs.

Despite the theoretical benefits of applying genetic testing, several factors hinder (immediate) application of the reviewed findings to clinical practice.

Firstly, except for one study [42], none of the studies have used replication cohorts. Also, most of the studies do not mention correction for multiple comparisons. Therefore, in order to exclude type I errors (i.e., false-positive findings), the study results should be confirmed in replication cohorts.

Secondly, most of these studies have been performed in Caucasian patients. The frequency of genetic polymorphisms is known to vary greatly between ethnicities. Therefore, an important and frequent SNP in one population might be much less relevant in another, limiting the external validity of pharmacogenetics studies to the ethnic group in which the studies were performed. To illustrate, Black and Hispanic lupus nephritis patients respond better to mycophenolate mofetil (MMF) than CYC, while MMF and CYC are equally effective in Asian and Caucasian patients [65].

Thirdly, the studies deal with genetic variants that occur only in a minority of patients. This means that large sample sizes will be required to detect relevant differences in clinical endpoints associated with these variants. Furthermore, all patients need to be genotyped to find the few patients that might benefit from adjustment of therapy. As AAV is a rare disease group, the required sample size will often be difficult to achieve, especially in a single-center setting.

Lastly, all of these studies were small observational cohort studies. In order to find the right dose adjustment or alternative drug and to confirm that this will achieve the desired improvement in treatment outcomes, randomized clinical trials (RCTs) should be performed. These studies should use relevant clinical outcomes to evaluate adjustment of therapy based on gene variants. Again, these studies require large sample sizes because of the large number of patients needed to genotype to find a patient requiring adjustment of therapy and the even larger number needed in to prevent occurrence of

an adverse effect. To illustrate, a large multi-center RCT investigating thiopurine dose reduction in inflammatory bowel disease patients carrying TPMT gene variants (the TOPIC trial) did not find a reduction of thiopurine toxicity for the intervention group in intention-to-treat analysis, even though a large reduction of toxicity was found within TPMT variant carriers. This is most likely because the majority of patients (90%) had a normal TPMT genotype and received a normal thiopurine dose even in the intervention group [21]. Because of the 10-fold increased risk of severe myelosuppression in the occasional homozygous variant carrier [20], TPMT pretesting may be relevant regardless. Because of small effects per genetic variant, combined with the low prevalence of both these gene variants and AAV, it might be most feasible to design one RCT in which multiple gene variants are measured simultaneously to inform treatment adjustment in the intervention group.

The studies reviewed in this paper have identified some interesting genetic variants that might be used to improve treatment outcomes after the previously mentioned hurdles have been overcome. On the other hand, based on the strength of current data, further confirmation of findings is needed before an RCT should be performed. Additional useful SNPs might be discovered by using data from genome-wide association studies, such as the one performed by Lyons et al. [66]. To illustrate, a GWAS study performed in RA patients identified several genetic variants associated with response to anti-TNF therapy [67]. The association of these gene variants with relevant clinical outcomes may be tested by genotyping samples from previously performed multi-center clinical trials. The on-going PEXIVAS trial, for example, may provide interesting samples and data to this end [68].

In summary, several studies have been performed regarding genetic polymorphisms in relation to efficacy and toxicity of CYC, RTX, PRED and AZA for treatment of AAV. Several challenges will need to be overcome for clinical application of these results, including independent replication of findings, identification of relevant SNPs per ethnic group, performing large enough multi-center studies to detect relevant clinical effects of SNPs that are present in a minority of patients, and performing large multi-center RCTs to evaluate personalized therapy based simultaneously on multiple SNPs to improve outcomes in this small group. Additional polymorphisms may be identified using GWAS data or samples from previously performed multi-center clinical trials.

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CONFLICT OF INTEREST
The authors declare no conflicts of interest.

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